review

Familial hypobetalipoproteinemia: a review

Gustav Schonfeld[1]

Washington University School of Medicine St. Louis, MO 63110

Abstract **We review the genetics and pathophysiology of familial hypobetalipoproteinemia (FHBL), a mildly symptomatic genetically heterogeneous autosomal trait. The minority of human FHBL is caused by truncation-specifying mutations of the *APOB* gene on chromosome 2. In seven families, linkage to chromosome 2 is absent, linkage is instead to chromosome 3 (3p21). In others, linkage is absent to both APOB and to 3p21. Apolipoprotein B-100 (apoB-100) levels are ~25% of normal, instead of the 50% expected based on the presence of one normal allele due to reduced rates of production. The presence of the truncating mutation seems to have a "dominant recessive" effect on apoB-100 secretion. Concentrations of apoB truncations in plasma differ by truncation but average at ~10% of normal levels. Lipoproteins bearing truncated forms of apoB are cleared more rapidly than apoB-100 particles. In contrast with apoB-100 particles cleared primarily in liver via the LDL receptor, most apoB truncation particles are cleared in renal proximal tubular cells via megalin. Since apoB defects cause a dysfunctional VLDL-triglyceride transport system, livers accumulate fat. Hepatic synthesis of fatty acids is reduced in compensation. Informational lacunae remain about genes affecting fat accumulation in liver, and the modulation of liver fat in the presence apoB truncation defects.**—Schonfeld, G. **Familial hypobetalipoproteinemia: a review.** ***J. Lipid Res.*** **2003.** 44: **878–883.**

Supplementary key words apolipoprotein B • microsomal triglyceride transfer protein • abetalipoproteinemia • low cholesterol • truncated apolipoprotein B • hepatosteatosis

Hypobetalipoproteinemia (HBL) is defined by <5th percentile plasma levels of total cholesterol or LDL cholesterol, or total apolipoprotein B (apoB), i.e., ~150, ~70, and ~50 mg/dl, respectively (1, 2). A number of different factors, such as diet and illness, can produce low apoB levels. Strict vegetarians (vegans) may have total cholesterol levels approaching the 5th percentile (3–5). Intestinal fat malabsorption such as that seen in sprue (chronic pancreatitis), severe liver disease, malnutrition, and hyperthyroidism may produce low apoB and cholesterol levels (6, 7). These are regarded as secondary causes of HBL. Primary causes include abetalipoproteinemia and chylomicron retention disease that segregate in families as autosomal recessive traits, and familial HBL (FHBL) that segregates as an autosomal dominant. Abetalipoproteinemia is due to a variety of genetic defects in microsomal triglyceride transfer protein (MTP), (8–10). The molecular basis of chylomicron retention disease is unknown (11). Both are characterized by an inability to produce chylomicrons in enterocytes, resulting in malabsorption of dietary fat and fat-soluble vitamins that produce severe sequelae in infancy and childhood such as "failure to thrive," anemia, acanthocytosis, ataxia, and retinitis pigmentosa. Early diagnosis and treatment can prevent many of the sequelae. By contrast, the overwhelming majority of subjects with FHBL are simple heterozygotes who are asymptomatic and yet have very low LDL cholesterol and apoB levels. Recently, however, it has been appreciated that a large proportion of subjects have nonalcoholic fatty livers (12–16). The long-term effects of this for health and longevity are unknown. Since the causes of FHBL are unknown in most cases (17), it is useful to identify additional relatives with low levels of LDL or apoB to make the diagnosis of FHBL, and to rule out the secondary causes of HBL. A few FHBL heterozygotes may have loose stools due to partial fat malabsorption, while homozygotes or compound heterozygotes may suffer from severe fat malabsorption, resembling patients with abetalipoproteinemia. As a rule though, even the severe forms of FHBL are less problematic for patients than abetalipoproteinemia (7, 18). Studies in humans, mouse models, and in cultured cells have helped to elucidate the genetic, pathophysiologic, and cellular/molecular bases of FHBL.

HUMAN STUDIES

Genetics

In most cases of HBL, the genetic cause(s) are not known (17). The best-characterized cases are due to mutations of the apoB gene. Most are missense and frame-shift mutations specifying the production of truncated proteins (19–21). The truncations are designated according to a centile nomenclature. Thus, the normal protein secreted from liver as

Manuscript received 17 January 2003 and in revised form 28 January 2003.

Published, JLR Papers in Press, March 16, 2003.
DOI 10.1194/jlr.R300002-JLR200

[1] To whom correspondence should be addressed.
e-mail: gschonfe@im.wustl.edu

part of VLDL particles and consisting of 4,536 amino acid residues is designated as apoB-100 (18). The normal intestinal variant associated with chylomicrons is apoB-48. The same gene located on chromosome 2 produces both apoB-48 and apoB-100. ApoB-48 results from a unique editing of apoB mRNA that converts codon 2,153 to a stop codon (22, 23). The editosome is present in the intestines of all mammals and in the livers of rodents, but not primates. Over 40 different abnormal truncations, some as short as apoB-2 and as long as apoB-89, have been described (14, 15, 24–35). Null alleles probably result in no translated mRNA (19, 27), and one FHBL family with an amino acid substitution has also been reported (36). ApoBs shorter than apoB-27.6 are usually absent from plasma due to their low production rates and rapid clearance (see below). Our group has identified 17 of the truncations reported in the literature.

FHBL appears to be genetically heterogeneous. Recently, we identified seven new families in whom no truncated forms of apoB were detectable in plasma, and FHBL segregated as a Mendelian dominant trait. A genome scan followed by linkage analysis revealed linkage to a susceptibility locus on chromosome 3p21 between markers D3S2407 and D3S1767 (37–39). In a third group of five families, genome scanning and linkage studies ruled out linkage to either chromosome 2 or chromosome 3p21 (G. Schonfeld, unpublished observations).

Metabolism

Three subfamilies of apoB-containing lipoproteins may circulate in the plasmas of subjects heterozygous for apoB truncations longer than apoB-27.6. For example, plasmas of subjects heterozygous for apoB-54.8 contain separate particles bearing apoB-100, apoB-48, and apoB-54.8 (35). ApoB-100 and apoB-48 are found in the population of VLDL-sized particles; however, the vast majority of apoB-100 in plasma circulates in association with LDL-sized particles. Diameters of lipoproteins bearing truncated forms of apoB vary in size directly with the lengths of the truncations, i.e., apoB-89-bearing particles have sizes and densities similar to normal VLDL and LDL. ApoB38.9-bearing particles have sizes intermediate between LDL and HDL, and densities similar to large HDLs (25, 40). Short truncations of apoB transport smaller numbers of triglyceride molecules than do the longer ones.

The average concentrations of apoB-100 in plasmas of heterozygotes would be expected to be ~50% of normal. In fact, levels are closer to ~25% of normal. This is due to production rates (determined in vivo) that are ~25% of normal production rates (41–43). The presence of the various forms of apoB on distinct particles permits concurrent metabolic studies to be performed in vivo to determine the kinetic parameters of apoB-100- and apoB truncation-bearing particles relative to each other in the same person. Levels of apoB truncations vary from about 10–30% of that of apoB-100 in the same person (i.e., ~3–9% of apoB concentration in normal subjects). This is due to a combination of low production rates and rapid clearance rates (44–47); however, the relative importance of production and clearance rates in setting plasma levels depends on the truncation in question. For example, the production rate of apoB-89-containing particles is only 15% lower than that of apoB-100 particles, but their clearance rate is more than twice normal due to the enhanced affinity of the interaction of apoB-89 with the LDL receptor (44). For apoB-75 particles, production is more impaired than for apoB-89, but clearance is still rapid due to enhanced interaction with the LDL receptor (48). The lipoproteins bearing apoB truncations shorter than apoB-75 are cleared very rapidly, mostly by the kidney, mediated by megalin/gp330 receptors located in proximal tubule cells (46, 47).

Hepatosteatosis

As a result of low rates of hepatic production of the normal lipid transporter protein, apoB-100, and the impaired capacities, particularly of short truncations of apoB to transport triglycerides, the VLDL export system for lipids is impaired. This would be expected to result in the accumulation of triglycerides and perhaps other lipid components of VLDL in liver. Indeed, several groups have reported on cases of hepatic steatosis detected by ultrasound or, in rare instances, by liver biopsy (12, 14, 15, 49). Recently, Schonfeld et al. have examined 22 individuals with various truncations of apoB ranging from apoB-4 to apoB-89 and 13 controls, using magnetic resonance spectroscopy (MRS) (16). Results are calculated from energy spectra and represent a precise noninvasive method for quantifying liver fat. The mean value for liver fat in FHBL subjects was five times that of controls (50, 51), suggesting that liver triglyceride contents are elevated. Since MRS as we use it cannot distinguish between the fatty acyl determinants associated with triglycerides, cholesteryl esters, and phospholipids, a theoretical possibility exists that all three of the major lipid moieties are elevated in humans. Indeed, it would not be surprising if all three moieties accumulated in liver since all are necessary for VLDL formation and all are part of VLDL particles; however, livers of mice with apoB truncations accumulate only triglycerides to a significant extent (see below). The differences in liver fat content between FHBL and controls could not be accounted for by caloric intake, dietary composition, indexes of total body fat and abdominal fat, or indexes of glucose tolerance and insulin sensitivity, suggesting that the *APOB* mutations per se were important contributors to fatty liver.

LESSONS FROM FHBL IN MICE AND CULTURED CELLS

Mice and cells

The production of apoB-containing lipoproteins has been studied in cell cultures using human HepG2 and rat Mc7777 hepatoma cell lines and in primary hepatocytes (52–57). In addition, several mice bearing different truncated forms of apoB have been produced that have helped to elucidate the pathophysiology of FHBL (58–61). Maeda's group has produced an apoB-81 mouse (62, 63), and Young's group has produced apoB-83 (59) and apoB-39 mice (59). We have produced an apoB-82-expressing HepG2 cell line

(64) and two apoB truncation-harboring mice by targeted homologous recombination in embryonic stem cells using the Cre-loxP system to excise any extraneous genomic sequences. The resulting apoB-38.9 (65) and apoB-27.6 (66) mice closely resemble their human counterparts with respect to the genomic sites of the mutations and phenotype.

Plasma lipoproteins

In contrast with humans, mouse livers produce not only apoB-100 and the apoB truncations, but also apoB-48. Thus, apoB-48-containing lipoproteins arise from both liver and intestine. The mice exhibit HBL with low levels of cholesterol and apoB, and as in human heterozygotes, three subfamilies of apoB-containing lipoproteins circulate in plasmas of $apoB^{38.9/+}$ and $apoB^{27.6/+}$ mice: those containing apoB-100, apoB-48, and the apoB truncation (65, 66). Plasma levels of apoB-100 and apoB-48 are about equal; levels of the truncations are much less. In the plasmas of $apoB^{38.9/38.9}$ and $apoB^{27.6/27.6}$ homozygous mice, only apoB truncation-containing lipoproteins circulate.

Lipoprotein metabolism

Studies of mice in vivo and in primary cultures of hepatocytes demonstrate that livers of $apoB^{38.9/+}$ mice secrete apoB-100 in small amounts, apoB-48 at ~10× the rate of apoB-100, and apoB-38.9 in equimolar amounts with apoB-48. In $apoB^{27.6/+}$ mice, the apoB-100 is secreted at ~1/10th the rate of apoB-48, but apoB-27.6 is secreted at ~5-fold the rate of apoB-48 (65, 66). Since plasma levels of both the apoB truncations are less than levels of apoB-48, the truncation-containing lipoproteins must be cleared more rapidly than apoB-48 lipoproteins. The more rapid clearance of the mutant apoBs is compatible with previous in vivo studies in humans and rabbits (44–47, 67, 68). Similarly, although more apoB-48 is secreted than apoB-100, plasma levels of apoB-48 lipoproteins are equal or less than levels of apoB-100 lipoproteins, suggesting that apoB-48 are cleared more rapidly than apoB-100.

In humans, the in vivo production rates of apoB-100 are ~25% of those of normal controls, instead of the 50% expected from the unhindered action of a single normal allele [see above and refs. (41–43)]. Similar "dominant negative" types of effects on apoB-100 secretion are seen in HepG2 cells engineered to express apoB-82 (64) and mice engineered to express apoB81 (62). $ApoB^{38.9/38.9}$ mice bred with $apoB^{100/100}$ mice produce $apoB^{38.9/100}$ offspring. Hepatocyte cultures of these animals synthesize only apoB-38.9 and apoB-100, but no apoB-48. Here too, apoB-100 is secreted at less than the expected 50% of controls. The dominant negative effect is not due to decreased rates of synthesis but rather due to reduced rates of secretion from cells, suggesting increased rates of intrahepatocytic degradation (Z. Chen and G. Schonfeld, unpublished observations). It would be important to know the cellular-molecular mechanism for this enhanced rate of degradation.

Hepatosteatosis

The low rate of synthesis and secretion of normal apoBs, the rapid clearance of truncation-containing lipoproteins, and the limited ability of the apoB-38.9 and -27.6 truncations to ferry triglycerides led us to predict that, just as in humans, our mice too would have fatty livers. Indeed, liver triglycerides were increased 1.5-fold and 3-fold in $apoB^{38.9/+}$ and $apoB^{38.9/38.9}$ mice, respectively, over age- and sex-matched $apoB^{+/+}$ wild types (64, 65). Similarly, liver triglycerides of $apoB^{27.6/+}$ and $apoB^{27.6/27.6}$ mice were increased 3-fold and 5-fold (66). Mean cholesterol contents were elevated by ~4%, but this was not statistically significant. Phospholipid contents were also not significantly elevated. The greater accumulation of triglycerides in the animals bearing the shorter truncation is compatible with the more severe defect in the transport capacity of the shorter truncation. It is not known, however, whether an inverse relationship exists between the lengths of apoB truncations over a wide range of lengths and the amount of fat accumulated in liver.

Feedback inhibition of hepatic triglyceride synthesis

We hypothesized that the *APOB* defect-induced disturbance of triglyceride transport could result in feedback inhibition of fatty acid and/or triglyceride synthesis in liver. Indeed, fatty acid synthesis measured as [^{14}C]acetate incorporation into triglycerides was decreased in a gene-dose dependent fashion (69). This was accompanied by decreases in hepatic mRNA levels for the transcription factor SREBP-1c that regulates several enzymes in the fatty acid synthetic pathway. The mRNA levels for two of the enzymes measured; fatty acid synthase and steroyl-CoA desaturase-1 were also lower. This feedback would tend to limit the amount of fat accumulated in the face of the *APOB* mutation-induced defect in the triglyceride export pathway. Thus, the amount of fat accumulated is under the control of several genes; the expression of the genes relative to each other could set hepatic triglyceride levels.

A region between apoB-38.9 and apoB-27.6 that supports embryogenesis

Heterozygous crosses between $apoB^{38.9/+} \times apoB^{38.9/+}$ are expected to yield offspring in the following proportions: 25% $apoB^{-/-}$, 25% $apoB^{38.9/38.9}$, and 50% $apoB^{+/38.9}$; however, such crosses in fact yield only ~12% $apoB^{38.9/38.9}$ offspring (65). Heterozygous crosses of $apoB^{27.6/+}$ yield only 3–4% $apoB^{27.6/27.6}$ homozygous offspring (66), similar to the yield of null homozygotes ($apoB^{0/0}$) in $apoB^{0/+} \times apoB^{0/-}$ crosses (58). Thus, the induction of a null mutation or the apoB-27.6 mutation in mouse *apob* results in high degrees of embryonic lethality for homozygotes. By contrast, $apoB^{38.9/+} \times apoB^{38.9/+}$ crosses do yield homozygotes that appear at least grossly normal, but in reduced numbers. This suggests that the first 27.6% of the N-terminal region contains very little, if any, sequence (or structure) able to support embryogenesis, but the next 11.3% (the stretch of sequence between apoB-27.6 and apoB-38.9) does contain such structures. The adequacy of this hypothesis was verified by making $apoB^{38.9/+} \times apoB^{27.6/+}$ crosses. The yields of the resultant compound heterozygotes ($apoB^{38.9/27.6}$) were nearly identical to the yields of apoB 38.9 homozygotes, i.e., apoB-38.9 was able to "rescue" apoB-27.6 fetuses (66).

ApoB-38.9 can support atherogenesis

ApoE$^{-/-}$ mice develop florid aortic atherosclerosis even while eating normal low-cholesterol and low-fat mouse chow (70, 71), and are widely used as animal models in studies of atherogenesis. ApoB-containing cholesterol-rich particles are responsible for the atherosclerosis in these animals. To assess whether particles containing apoB-38.9 rather than the normal variants apoB-48 or apoB-100 could support the development of aortic lesions, apoB$^{38.9/38.9}$ mice were crossed with apoE$^{-/-}$ mice. The resultant doubly homozygous apoB$^{38.9/38.9}$/apoE$^{-/-}$ mice developed just as much atherosclerosis as the apoB$^{+/+}$/apoE$^{-/-}$ mice did. Thus, the first 38.9% of the N-terminal end of apoB contains sufficient structure for lesion formation.

SUMMARY

In summary, the reported studies have elucidated the genetic bases of some forms of FHBL, and the pathophysiology for the low levels of apoB-containing lipoproteins in humans with FHBL and mice engineered to resemble FHBL. They have also provided new information on some of the functional domains of apoB in lipid transport, embryogenesis, and atherogenesis.

This work was supported by National Institutes of Health Grants RO1 HL-59515 and R37 HL-42460 and by a grant from the Albert and Helen Wolf Charitable Foundation. The author is grateful to our patients, to nurses Sherry Banez-Mueth and Jacqueline Dudley, and to co-workers Zhang Chen, Xiaobo Lin, Pin Yue, Maurizio Averua, Bo Yuan, Rosalind Neuman, Daniela Gerhard, JingShi Wu, Klaus Parhofer, Elaine Krul, Aiit Suvastava, and D. Yablonskig.

REFERENCES

1. Heiss, G., I. Tamir, C. E. Davis, H. A. Tyroler, B. M. Rifkand, G. Schonfeld, D. Jacobs, and I. D. Frantz, Jr. 1980. Lipoprotein-cholesterol distributions in selected North American populations: the lipid research clinics program prevalence study. *Circulation.* **61:** 302–315.
2. Contois, J. H., J. R. McNamara, C. J. Lammi-Keefe, P. W. Wilson, T. Massov, and E. J. Schaefer. 1996. Reference intervals for plasma apolipoprotein B determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. *Clin. Chem.* **42:** 515–523.
3. Burslem, J., G. Schonfeld, M. A. Howald, S. W. Weidman, and J. P. Miller. 1978. Plasma apoprotein and lipoprotein lipid levels in vegetarians. *Metabolism.* **27:** 711–719.
4. Sacks, F. M., W. P. Castelli, A. Donner, and E. H. Kass. 1975. Plasma lipids and lipoproteins in vegetarians and controls. *N. Engl. J. Med.* **292:** 1148–1151.
5. Burr, M. L., C. J. Bates, A. M. Fehily, and A. S. St Leger. 1981. Plasma cholesterol and blood pressure in vegetarians. *J. Hum. Nutr.* **35:** 437–441.
6. 2001. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA.* **285:** 2486–2497.
7. Kane, J. P., and R. J. Havel. 1995. Disorders of the biogenesis and secretion of lipoproteins containing the B apolipoproteins. *In* The Metabolic and Molecular Bases of Inherited Disease. McGraw-Hill Inc., New York. 1853–1885.
8. Berriot-Varoqueaux, N., L. P. Aggerbeck, M. Samson-Bouma, and J. R. Wetterau. 2000. The role of the microsomal triglygeride transfer protein in abetalipoproteinemia. *Annu. Rev. Nutr.* **20:** 663–697.
9. Kayden, H. J. 2001. The genetic basis of vitamin E deficiency in humans. *Nutrition.* **17:** 797–798.
10. Wetterau, J. R., L. P. Aggerbeck, M. E. Bouma, C. Eisenberg, A. Munck, M. Hermier, J. Schmitz, G. Gay, D. J. Rader, and R. E. Gregg. 1992. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. *Science.* **258:** 999–1001.
11. Berriot-Varoqueaux, N., A. H. Dannoura, A. Moreau, N. Verthier, A. Sassolas, G. Cadiot, A. Lachaux, A. Munck, J. Schmitz, L. P. Aggerbeck, and M. E. Samson-Bouma. 2001. Apolipoprotein B-48 glycosylation in abetalipoproteinemia and Anderson's disease. *Gastroenterology.* **121:** 1101–1108.
12. Tarugi, P., and A. Lonardo. 1997. Heterozygous familial hypobetalipoproteinemia associated with fatty liver. *Am. J. Gastroenterol.* **92:** 1400–1402.
13. Tarugi, P., A. Lonardo, G. Ballarini, L. Erspamer, E. Tondelli, S. Bertolini, and S. Calandra. 2000. A study of fatty liver disease and plasma lipoproteins in a kindred with familial hypobetalipoproteinemia due to a novel truncated form of apolipoprotein B (APO B-54.5). *J. Hepatol.* **33:** 361–370.
14. Tarugi, P., A. Lonardo, G. Ballarini, A. Grisendi, M. Pulvirenti, A. Bagni, and S. Calandra. 1996. Fatty liver in heterozygous hypobetalipoproteinemia caused by a novel truncated form of apolipoprotein B. *Gastroenterology.* **111:** 1125–1133.
15. Tarugi, P., A. Lonardo, C. Gabelli, F. Sala, G. Ballarini, I. Cortella, L. Previato, S. Bertolini, R. Cordera, and S. Calandra. 2001. Phenotypic expression of familial hypobetalipoproteinemia in three kindreds with mutations of apolipoprotein B gene. *J. Lipid Res.* **42:** 1552–1561.
16. Schonfeld, G., B. W. Patterson, D. A. Yablonskiy, T. S. K. Tanoli, M. Averna, N. Elias, P. Yue, and J. Ackerman. 2003. Fatty liver in familial hypobetalipoproteinemia: triglyceride assembly into VLDL particles is affected by the extent of hepatic steatosis. *J. Lipid Res.* **44:** 470–478.
17. Wu, J., J. Kim, Q. Li, P. Y. Kwok, T. G. Cole, B. Cefalu, M. Averna, and G. Schonfeld. 1999. Known mutations of apoB account for only a small minority of hypobetalipoproteinemia. *J. Lipid Res.* **40:** 955–959.
18. Havel, R. J., and J. P. Kane, editors. 1995. Structure and Metabolism of Plasma Lipoproteins. 7th edition. The Metabolic Basis of Inherited Disease. McGraw-Hill Co., New York.
19. Schonfeld, G. 1995. The hypobetalipoproteinemias. *Annu. Rev. Nutr.* **15:** 23–34.
20. Farese, R. V., Jr., M. F. Linton, and S. G. Young. 1992. Apolipoprotein B gene mutations affecting cholesterol levels. *J. Intern. Med.* **231:** 643–652.
21. Linton, M. F., R. V. Farese, Jr., and S. G. Young. 1993. Familial hypobetalipoproteinemia. *J. Lipid Res.* **34:** 521–541.
22. Davidson, N. O., and G. S. Shelness. 2000. Apolipoprotein B: mRNA editing, lipoprotein assembly, and presecretory degradation. *Annu. Rev. Nutr.* **20:** 169–193.
23. Chen, L., and L. Chan. 1996. Control of apolipoprotein B mRNA editing: implication of mRNA dynamics at various maturation stages. *J. Theor. Biol.* **183:** 391–407.
24. Pulai, J. I., H. Zakeri, P. Y. Kwok, J. H. Kim, J. Wu, and G. Schonfeld. 1998. Donor splice mutation (665 + 1 G_T) in familial hypobetalipoproteinemia with no detectable apoB truncation. *Am. J. Med. Genet.* **80:** 218–220.
25. Krul, E. S., M. Kinoshita, P. Talmud, S. E. Humphries, S. Turner, A. C. Goldberg, K. Cook, E. Boerwinkle, and G. Schonfeld. 1989. Two distinct truncated apolipoprotein B species in a kindred with hypobetalipoproteinemia. *Arteriosclerosis.* **9:** 856–868.
26. Talmud, P., L. King-Underwood, E. Krul, G. Schonfeld, and S. Humphries. 1989. The molecular basis of truncated forms of apolipoprotein B in a kindred with compound heterozygous hypobetalipoproteinemia. *J. Lipid Res.* **30:** 1773–1779.
27. Welty, F. K., K. A. Guida, and J. J. Andersen. 2001. Donor splice-site mutation (210+1G_C) in the ApoB gene causes a very low level of ApoB-100 and LDL cholesterol. *Arterioscler. Thromb. Vasc. Biol.* **21:** 1864–1865.
28. Welty, F. K., S. T. Hubl, V. R. Pierotti, and S. G. Young. 1991. A truncated species of apolipoprotein B (B67) in a kindred with familial hypobetalipoproteinemia. *J. Clin. Invest.* **87:** 1748–1754.
29. Welty, F. K., J. Ordovas, E. J. Schaefer, P. W. Wilson, and S. G.

Young. 1995. Identification and molecular analysis of two apoB gene mutations causing low plasma cholesterol levels. *Circulation.* **92:** 2036–2040.
30. Hardman, D. A., C. R. Pullinger, R. L. Hamilton, J. P. Kane, and M. J. Malloy. 1991. Molecular and metabolic basis for the metabolic disorder normotriglyceridemic abetalipoproteinemia. *J. Clin. Invest.* **88:** 1722–1729.
31. Pullinger, C. R., E. Hillas, D. A. Hardman, G. C. Chen, J. M. Naya-Vigne, J. A. Iwasa, R. L. Hamilton, J. M. Lalouel, R. R. Williams, and J. P. Kane. 1992. Two apolipoprotein B gene defects in a kindred with hypobetalipoproteinemia, one of which results in a truncated variant, apoB-61, in VLDL and LDL. *J. Lipid Res.* **33:** 699–710.
32. Young, S. G., C. R. Pullinger, B. R. Zysow, H. Hofmann-Radvani, M. F. Linton, R. V. Farese, Jr., J. F. Terdiman, S. M. Snyder, S. M. Grundy, G. L. Vega, et al. 1993. Four new mutations in the apolipoprotein B gene causing hypobetalipoproteinemia, including two different frameshift mutations that yield truncated apolipoprotein B proteins of identical length. *J. Lipid Res.* **34:** 501–507.
33. Talmud, P. J., C. Converse, E. Krul, L. Huq, G. G. McIlwaine, J. J. Series, P. Boyd, G. Schonfeld, A. Dunning, and S. Humphries. 1992. A novel truncated apolipoprotein B (apo B55) in a patient with familial hypobetalipoproteinemia and atypical retinitis pigmentosa. *Clin. Genet.* **42:** 62–70.
34. Talmud, P. J., E. S. Krul, M. Pessah, G. Gay, G. Schonfeld, S. E. Humphries, and R. Infante. 1994. Donor splice mutation generates a lipid-associated apolipoprotein B-27.6 in a patient with homozygous hypobetalipoproteinemia. *J. Lipid Res.* **35:** 468–477.
35. Wagner, R. D., E. S. Krul, J. Tang, K. G. Parhofer, K. Garlock, P. Talmud, and G. Schonfeld. 1991. ApoB-54.8, a truncated apolipoprotein found primarily in VLDL, is associated with a nonsense mutation in the apoB gene and hypobetalipoproteinemia. *J. Lipid Res.* **32:** 1001–1011.
36. Burnett, J. R., J. Shan, B. A. Miskie, A. J. Whitfield, J. Yuan, K. Tran, C. J. McKnight, R. A. Hegele, and Z. Yao. 2003. A novel nontruncating *APOB* gene mutation, R463W, causes familial hypobetalipoproteinemia. *J. Biol. Chem.* **278:** 13442-13452.
37. Pulai, J. I., R. J. Neuman, A. W. Groenewegen, J. Wu, and G. Schonfeld. 1998. Genetic heterogeneity in familial hypobetalipoproteinemia: linkage and nonlinkage to the apoB gene in Caucasian families. *Am. J. Med. Genet.* **76:** 79–86.
38. Yuan, B., R. Neuman, S. H. Duan, J. L. Weber, P. Y. Kwok, N. L. Saccone, J. S. Wu, K. Y. Liu, and G. Schonfeld. 2000. Linkage of a gene for familial hypobetalipoproteinemia to chromosome 3p21.1–22. *Am. J. Hum. Genet.* **66:** 1699–1704.
39. Neuman, R. J., B. Yuan, D. S. Gerhard, K. Y. Liu, P. Yue, S. Duan, M. Averna, and G. Schonfeld. 2002. Replication of linkage of familial hypobetalipoproteinemia to chromosome 3p in six kindreds. *J. Lipid Res.* **43:** 407–415.
40. Groenewegen, W. A., M. R. Averna, J. Pulai, E. S. Krul, and G. Schonfeld. 1994. Apolipoprotein B-38.9 does not associate with apo(a) and forms two distinct HDL density particle populations that are larger than HDL. *J. Lipid Res.* **35:** 1012–1025.
41. Aguilar-Salinas, C. A., P. H. Barrett, K. G. Parhofer, S. G. Young, D. Tessereau, J. Bateman, C. Quinn, and G. Schonfeld. 1995. Apoprotein B-100 production is decreased in subjects heterozygous for truncations of apoprotein B. *Arterioscler. Thromb. Vasc. Biol.* **15:** 71–80.
42. Elias, N., B. W. Patterson, and G. Schonfeld. 1999. Decreased production rates of VLDL triglycerides and ApoB-100 in subjects heterozygous for familial hypobetalipoproteinemia. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2714–2721.
43. Elias, N., B. W. Patterson, and G. Schonfeld. 2000. In vivo metabolism of ApoB, ApoA-I, and VLDL triglycerides in a form of hypobetalipoproteinemia not linked to the ApoB gene. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1309–1315.
44. Parhofer, K. G., P. H. Barrett, D. M. Bier, and G. Schonfeld. 1992. Lipoproteins containing the truncated apolipoprotein, Apo B-89, are cleared from human plasma more rapidly than Apo B-100-containing lipoproteins in vivo. *J. Clin. Invest.* **89:** 1931–1937.
45. Parhofer, K. G., A. Daugherty, M. Kinoshita, and G. Schonfeld. 1990. Enhanced clearance from plasma of low density lipoproteins containing a truncated apolipoprotein, apoB-89. *J. Lipid Res.* **31:** 2001–2007.
46. Chen, Z., J. E. Saffitz, M. A. Latour, and G. Schonfeld. 1999. Truncated apo B-70.5-containing lipoproteins bind to megalin but not the LDL receptor. *J. Clin. Invest.* **103:** 1419–1430.
47. Zhu, X. F., D. Noto, R. Seip, A. Shaish, and G. Schonfeld. 1997. Organ loci of catabolism of short truncations of apoB. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1032–1038.
48. Krul, E. S., K. G. Parhofer, P. H. Barrett, R. D. Wagner, and G. Schonfeld. 1992. ApoB-75, a truncation of apolipoprotein B associated with familial hypobetalipoproteinemia: genetic and kinetic studies. *J. Lipid Res.* **33:** 1037–1050.
49. Lonardo, A., P. Tarugi, G. Ballarini, and A. Bagni. 1998. Familial heterozygous hypobetalipoproteinemia, extrahepatic primary malignancy, and hepatocellular carcinoma. *Dig. Dis. Sci.* **43:** 2489–2492.
50. Schonfeld, G., J. Ackerman, and D. Yablonskiy. 2001. Fatty liver in familial hypobetalipoproteinemia (FHBL) (Abstract). *Arterioscler. Thromb. Vasc. Biol.* **21:** 656.
51. Yablonskiy, D. A., J. J. Ackerman, and G. Schonfeld. 2001. Liver-fat content quantification by MR spectroscopy in patients with apoB truncation-containing lipoproteins (Abstract). *Radiology.* **221:** 496.
52. Davis, R. A., and T. Y. Hui. 2001. 2000 George Lyman Duff Memorial Lecture: atherosclerosis is a liver disease of the heart. *Arterioscler. Thromb. Vasc. Biol.* **21:** 887–898.
53. Ginsberg, H. N. 1995. Synthesis and secretion of apolipoprotein B from cultured liver cells. *Curr. Opin. Lipidol.* **6:** 275–280.
54. Gordon, D. A., and H. Jamil. 2000. Progress towards understanding the role of microsomal triglyceride transfer protein in apolipoprotein-B lipoprotein assembly. *Biochim. Biophys. Acta.* **1486:** 72–83.
55. Olofsson, S. O., P. Stillemark-Billton, and L. Asp. 2000. Intracellular assembly of VLDL: two major steps in separate cell compartments. *Trends Cardiovasc. Med.* **10:** 338–345.
56. Shelness, G. S., M. F. Ingram, X. F. Huang, and J. A. DeLozier. 1999. Apolipoprotein B in the rough endoplasmic reticulum: translation, translocation and the initiation of lipoprotein assembly. *J. Nutr.* **129:** 456S–462S.
57. Tran, K., Y. Wang, C. J. DeLong, Z. Cui, and Z. Yao. 2000. The assembly of very low density lipoproteins in rat hepatoma McA-RH7777 cells is inhibited by phospholipase A2 antagonists. *J. Biol. Chem.* **275:** 25023–25030.
58. Homanics, G. E., T. J. Smith, S. H. Zhang, D. Lee, S. G. Young, and N. Maeda. 1993. Targeted modification of the apolipoprotein B gene results in hypobetalipoproteinemia and developmental abnormalities in mice. *Proc. Natl. Acad. Sci. USA.* **90:** 2389–2393.
59. Kim, E., P. Ambroziak, M. M. Veniant, R. L. Hamilton, and S. G. Young. 1998. A gene-targeted mouse model for familial hypobetalipoproteinemia. Low levels of apolipoprotein B mRNA in association with a nonsense mutation in exon 26 of the apolipoprotein B gene. *J. Biol. Chem.* **273:** 33977–33984.
60. Raabe, M., E. Kim, M. Veniant, L. B. Nielsen, and S. G. Young. 1998. Using genetically engineered mice to understand apolipoprotein-B deficiency syndromes in humans. *Proc. Assoc. Am. Physicians.* **110:** 521–530.
61. Veniant, M. M., E. Kim, S. McCormick, J. Boren, L. B. Nielsen, M. Raabe, and S. G. Young. 1999. Insights into apolipoprotein B biology from transgenic and gene-targeted mice. *J. Nutr.* **129:** 451S–455S.
62. Srivastava, R. A., L. Toth, N. Srivastava, M. E. Hinsdale, N. Maeda, A. B. Cefalu, M. Averna, and G. Schonfeld. 1999. Regulation of the apolipoprotein B in heterozygous hypobetalipoproteinemic knock-out mice expressing truncated apoB, B81. Low production and enhanced clearance of apoB cause low levels of apoB. *Mol. Cell. Biochem.* **202:** 37–46.
63. Farese, R. V., Jr., A. Garg, V. R. Pierotti, G. L. Vega, and S. G. Young. 1992. A truncated species of apolipoprotein B, B-83, associated with hypobetalipoproteinemia. *J. Lipid Res.* **33:** 569–577.
64. Srivastava, R. A., N. Srivastava, M. Averna, A. B. Cefalu, and G. Schonfeld. 1999. Molecular bases of low production rates of apolipoprotein B-100 and truncated apoB-82 in a mutant HepG2 cell line generated by targeted modification of the apolipoprotein B gene. *J. Lipid Res.* **40:** 901–912.
65. Chen, Z., R. L. Fitzgerald, M. R. Averna, and G. Schonfeld. 2000. A targeted apolipoprotein B-38.9-producing mutation causes fatty livers in mice due to the reduced ability of apolipoprotein B-38.9 to transport triglycerides. *J. Biol. Chem.* **275:** 32807–32815.
66. Chen, Z., R. L. Fitzgerald, and G. Schonfeld. 2002. Hypobetalipoproteinemic mice with a targeted apolipoprotein (Apo) B-27.6-specifying mutation: in vivo evidence for an important role of

amino acids 1254–1744 of ApoB in lipid transport and metabolism of the apoB-containing lipoprotein. *J. Biol. Chem.* **277:** 14135–14145.
67. Welty, F. K., A. H. Lichtenstein, P. H. Barrett, G. G. Dolnikowski, J. M. Ordovas, and E. J. Schaefer. 1997. Decreased production and increased catabolism of apolipoprotein B-100 in apolipoprotein B-67/B-100 heterozygotes. *Arterioscler. Thromb. Vasc. Biol.* **17:** 881–888.
68. Welty, F. K., A. H. Lichtenstein, P. H. Barrett, G. G. Dolnikowski, J. M. Ordovas, and E. J. Schaefer. 1997. Production of apolipoprotein B-67 in apolipoprotein B-67/B-100 heterozygotes: technical problems associated with leucine contamination in stable isotope studies. *J. Lipid Res.* **38:** 1535–1543.
69. Lin, X., G. Schonfeld, P. Yue, and Z. Chen. 2002. Hepatic fatty acid synthesis is suppressed in mice with fatty livers due to targeted apolipoprotein B38.9 mutation. *Arterioscler. Thromb. Vasc. Biol.* **22:** 476–482.
70. Dansky, H. M., S. A. Charlton, J. L. Sikes, S. C. Heath, R. Simantov, L. F. Levin, P. Shu, K. J. Moore, J. L. Breslow, and J. D. Smith. 1999. Genetic background determines the extent of atherosclerosis in ApoE-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **19:** 1960–1968.
71. Calleja, L., M. A. Paris, A. Paul, E. Vilella, J. Joven, A. Jimenez, G. Beltran, M. Uceda, N. Maeda, and J. Osada. 1999. Low-cholesterol and high-fat diets reduce atherosclerotic lesion development in ApoE-knockout mice. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2368–2375.